

### **Amendments to the Specification**

Please add the following new paragraphs after page 1, line 21, which correspond to the Summary of the Invention and the Brief Description of the Drawings:

### **SUMMARY OF THE INVENTION**

The invention provides a peptide having at least about 75% amino acid homology with the sequence shown in SEQ ID No: 2. The peptide of the invention also can include an amino acid homology with the sequence shown in SEQ ID No: 2 corresponding to at least about 80%, at least about 85%, at least about 90% or can include the amino acid sequence shown in SEQ ID No: 2. The peptide of the invention can further include an amino acid sequence selected from the sequences shown in SEQ ID Nos: 1, 3 or 4-6.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1. Analysis of proteins found in the culture supernatant of *L. fermentum* BR11 grown in MRS broth. Growth of *L. fermentum* BR11 was monitored over 24-h by optical density measurements at 600nm. At various time points, indicated by a number in a circle, aliquots were taken, centrifuged and the supernatant filtered and precipitated with 5% TCA. The equivalent of 225- $\mu$ l of culture supernatant was analysed by SDS-PAGE followed by staining with Coomassie brilliant blue G-250. The arrow indicates Sep.

Figure 2. Expression and subcellular location of a His<sub>6</sub>-Sep protein in *L. fermentum* BR11, *L. rhamnosus* GG and *L. lactis* MG1363. Above shows the arrangement of the constructs which were either integrated into the *L. fermentum* BR11 chromosome (Sep-6xHis-Sep and BspA-6xHis-Sep) or introduced into *L. rhamnosus* GG or *L. lactis* MG1363 on the pGh9:ISS1 plasmid (Sep-6xHis-Sep only). Below shows Western blot detection of fusion proteins in cell extracts and in the supernatant using an anti-His<sub>5</sub> antibody. For the diagrams the *sep* terminator (*Tsep*) and DNA encoding the Sep secretion signal (*ssSep*), BspA secretion signal (*ssBspA*) and His<sub>6</sub> (grey box) are indicated. The DNA region which is the site of single crossover homologous recombination into either the *sep* or *bspA* loci of *L. fermentum* BR11 is spotted and below is marked with a cross. Sizes of molecular mass markers are indicated in kDa on the left. The lanes containing cell extracts prepared by

boiling in 2x SDS-loading dye (SDS), by sonication (son) and with 5M LiCl (LiCl) and the precipitated supernatant fractions (SN) are indicated. The amount of cells or medium loaded in each lane are the equivalent to 500µl (SDS), 50µl (son), 160µl (LiCl) and 675µl (SN) of culture.

Figure 3. Expression and secretion of human E-cadherin fusion protein by *L. fermentum* BR11. Above shows the arrangement of the constructs which were introduced into *L. fermentum* BR11 (Sep-6xHis-Ecad and BspA-6xHis-Ecad). Below shows Western blot detection of fusion proteins in cell extracts and in the supernatant using an anti-His<sub>5</sub> antibody (C[right side]). For the diagrams the *bspA* terminator (T *bspA*) and DNA encoding the Sep secretion signal (ssSep), BspA secretion signal (ssBspA) and His<sub>6</sub> (grey box) are indicated. The DNA region which is the site of single crossover homologous recombination into either the *sep* or *bspA* loci of *L. fermentum* BR11 is spotted and below is marked with a cross. The sizes of the molecular mass markers are indicated in kDA on the left. The lanes containing cell extracts prepared by boiling in 2X SDS-loading dye (SDS), by sonication (son) and with 5M LiCl (LiCl) and the precipitated supernatant fractions (SN) are indicated. The amount of cells or medium loaded in each lane are the equivalent to 500µl (SDS), 50µl (son), 160µl (LiCl) and 675µl (SN) of culture. For the Western blot in part C, the equivalent of 1.2-ml of culture supernatant from *L. fermentum* BR11 parent (BR11) or *L. fermentum* containing BspA-6xHis-Ecad (BspA-6xHis-Ecad) was located in each lane.

Figure 4. Expression of human vitronectin using the expression and secretion signals of Sep. The lanes containing cell extracts (C) prepared by boiling cells in 2x SDS-PAGE loading buffer and the precipitated supernatant fractions (S) are indicated. The amount of cells or medium loaded in each lane is the equivalent to 1ml (C) and 900µl (S) of culture.

Figure 5. Features of the pSep511sec plasmid used for expression and secretion of Ply511 in lactic acid bacteria. The origin of the temperature sensitive origin of replication (Ts) of pGh9::ISS1 is indicated while the direction of the erythromycin resistance (Em<sup>R</sup>) marker gene is also indicated by an arrow. The Sep-6xHis-Ply511 expression construct cloned into pGh9::ISS1 is shown with an arrow head indicating the likely *sep* promoter, a hatched box indicating Sep secretion signal (ssSep), a grey box indicating the His<sub>6</sub> epitope encoding DNA and a lollipop indicating the *bspA* operon terminator (T*bspA*). At the bottom

of the figure is the nucleotide and translated amino acid sequence of the junction between the Sep secretion signal and the Ply511 encoding DNA. The vertical arrow indicates the signal peptide cleavage site while the horizontal arrow indicates the start of Ply511.

Figure 6. Analysis of expression, secretion and activity of Ply511 produced by *Lactobacillus* spp. and *L. lactis*. (A) Western blot detection of proteins in the cell C) and supernatant (S) of lactic acid bacteria containing pSep<sub>511</sub>sec using anti His5-HRP conjugate. The amounts of cell extract or medium loaded in each lane are the equivalent to 500µl and 675µl of culture, respectively. (B) Detection of bacteriolytic activity of lactic acid bacterial supernatant fractions using renaturing SDS-PAGE with autoclaved *L. monocytogenes* as the substrate. For each strain the (-) lane indicates either pGH9::ISS1 containing (*L. lactis*, *L. fermentum* and *L. rhamnosus*) or wild-type (*L. plantarum*) strains while the (+) lane indicates pSep<sub>511</sub>sec containing strains.

Figure 7. Cell wall lytic activity of strains of lactic acid bacteria grown on buffered agar medium containing autoclaved *L. monocytogenes* cells. *L. lactis* were grown on buffered GM17E while *Lactobacillus* spp. strains containing plasmids were grown on buffered MRS with erythromycin while *L. plantarum* wild-type was grown on buffered MRS without erythromycin.

Figure 8. Sep LysM domain amino acid sequence (SEQ ID NO:1).

Figure 9. Sep C-terminal (apf) domain amino acid sequence (SEQ ID NO:2).

Figure 10. Sep glutamine-rich region (SEQ ID NO:3).

Figure 11. Sep secretion signal amino acid sequence (SEQ ID NO:4).

Figure 12. Sep amino acid sequence (SEQ ID NO:5).

Figure 13. Sep amino acid sequence including secretion signal sequence ((SEQ ID NO:6).

Figure 14. Sep secretion signal nucleotide sequence (SEQ ID NO:7). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 15. Sep LysM domain nucleotide sequence (SEQ ID NO:8). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 16. Sep glutamine-rich region nucleotide sequence (SEQ ID NO:9). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 17. Sep C-terminal nucleotide sequence (SEQ ID NO:10). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 18. Sep nucleotide sequence (SEQ ID NO:11). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 19. Sep nucleotide sequence including secretion signal coding sequence (SEQ ID NO:12). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 20. Sep secretion signal backtranslation sequence (SEQ ID NO:13). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 21. Sep LysM domain signal backtranslation sequence (SEQ ID NO:14). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 22. Sep glutamine-rich region signal backtranslation sequence (SEQ ID NO:15). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 23. Sep C-terminal signal backtranslation sequence (SEQ ID NO:16). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 24. Sep backtranslation sequence (SEQ ID NO:17). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 25. Sep including secretion signal backtranslation sequence (SEQ ID NO:18). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 26. Nucleotide sequence of 310-bp immediately upstream of *sep* containing a possible *sep* promoter (putative -35 and -10 recognition hexamers are indicated as shaded letters and bold letters respectively [note one -35 consensus also may be a -10 consensus]; TG motifs upstream of the putative -10 consensus hexamers are italicized; the *sep* ribosome binding site is underlined (SEQ ID NO:19). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Figure 27. Nucleotide sequence of 150-bp immediately downstream of *sep* containing *sep* transcription terminator (indicated as converging arrows above the sequence (SEQ ID NO:20). **IUB Mixed Base Codes:** R=AG Y=CT M=AC K=GT S=GC W=AT H=ACT B=GCT V=AGC D=AGT N=AGCT.

Please delete the paragraphs beginning at page 23, line 16 through page 27, line 27, which corresponds to a section entitled "Brief description of the drawings."